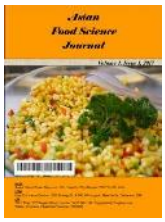

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Spectral Studies and Photo-Sensitized Oxidation of Melon Seed Oil

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Authors' contributions

The work was carried out in collaboration between both authors. Author SSA designed the study, wrote the protocol and wrote the first draft of the manuscript. Author AKI managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Purified samples of melon seed (*Citrullus lanatus*) oil extracted from melon seeds obtained from a local market in Yenagoa, Bayelsa State, Nigeria were analyzed by FTIR, ^{1}H NMR, ^{13}C and DEPT-135 NMR. The frequency data shows the oil to contain unsaturated acyl chains in its triacylglycerols. The FTIR shows stretch vibrations of the cis-olefin double bond and the ester carbonyl at 1748 cm^{-1}. The ^{1}H NMR confirms the presence of allylic, 1.99-2.04, bis-allylic, 2,726-2.753 vinylic, 5.265-5.328 and glyceryl 4.097-4.238 (Sn-1,3) and 5.421-5.328 (Sn-2) protons. These are further confirmed by data from carbon-13 NMR; signals between 27.17 and 27.18 (allylic carbons); the signal at 25.2 (bis-allylic carbons); signals between 120 and 130 (vinlylic carbons), signals at 68.91 (Sn-1,3) and 62.07 (Sn-2) (glyceryl carbons). Some deductions of the ^{13}C NMR data were further confirmed by data from the DEPT-135. Sensitized oxidation of a sample of the oil in the presence of methylene blue and monitored by thin layer chromatography and chemical analysis shows the formation of peroxide. The results from the sensitized photooxidation indicate that exposure of the oil in the presence of sensitizers such as chromophoric impurities is likely to reduce the shelf-life of the oil.

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Keywords: Photooxidation; spectra; melon seed oil; sensitizer; fatty acids.

1. INTRODUCTION

The egusi (melon plant) is an annual, herbaceous, monoecious plant with a non-climbing creeping habit [1]. The plant thrives in tropical, sub-tropical, arid deserts and temperate locations and belongs to the *Cucurbitaceae* family that has a tremendous genetic diversity, extending to vegetative and reproductive characteristics.

Various authors have shown that the oil content of the seed varies between 40 and 50% [2,3], depending on bioclimatic region of production. In West Africa, a region where soups are an integral to life, egusi melon (*Citrullus lanatus*) seeds are a major soup ingredient and a common component of daily meals. On the average, the oil has the following fatty acid composition: Lauric acid (0.21%); Myristic acid (0.78%); Palmitic acid (13.4%); Stearic acid (13.71%); Oleic acid (14.50%); Linoleic acid (56.94%) and Linolenic acid (0.46%) [4].

Fourier transform infra-red (FTIR), proton (^{1}H NMR) and carbon-13(^{13}C NMR) spectroscopic methods are non-destructive techniques for the study of edible oils and fats. Data obtained from these methods reveal some valuable information such as the relative proportions of acyl groups, the ester carbonyl functional group, acyl chain length and degree of unsaturation. As a result, these methods are used to characterize, authenticate and assess the quality of edible oils. Some authors have used data from FTIR and NMR to determine the quality parameters of oils such as iodine value, peroxide value and saponification value [5,6].

FTIR and NMR data reveal structural features the various groups in the acyl chains such as the glyceride ester carbonyl (3471.21 cm^{-1}) the cis-olefin (CH) of double bonds (3010-2800 cm^{-1}), the carbonyl group (1746.08 cm^{-1}). The nature of acyl chains presents whether linoleic, oleic or linolenic can also be detected from these data. The various hydrogen atoms in the acyl chains can be deduced from on a high resolution ^{1}H NMR spectrum. For example, the allylic hydrogen atoms of the oleyl and linoleyl groups are observed between 1.94-2.14 ppm, the bis-allylic hydrogen atoms of the linoleyl and linolenyl groups are usually observed between 2.70-2.84 ppm, the Sn-1,3 hydrogens of the glyceryl group (4.10-4.32 ppm), the Sn-2 hydrogens of the glyceryl group (5.20-5.26 ppm) while the vinylic protons are usually observed between 5.26-5.40 ppm [7].

The deterioration of vegetable oils can also be monitored through data obtained from FTIR and NMR spectroscopy. Usually, the onset of rancidity (hydrolytic and oxidative) is accompanied by changes in the structures of acyl chains in the TAGs. For example, oxidative deterioration is accompanied by the formation of the hydroperoxide group, that shows absorptions around 3444 cm^{-1} in the FTIR spectrum and signals between 8.6-8.3 ppm in the ^{1}H NMR spectrum. On the other hand, hydrolytic rancidity produces free fatty acids which absorb at 1710 cm^{-1} [8].

2. MATERIALS AND METHODS

The materials used in this work are melon seed oil, silica gel 60-200 mesh (Burgoyne Bridges, India, methylene blue (M&B), dichloromethane (Sigma Aldrich), hexane (60-80°C) methanol and ethyl acetate (BDH).

2.1 Sample Collection

The melon seeds used for melon oil extraction were obtained from local suppliers in Yenagoa, Bayelsa State, Nigeria.

2.2 Oil Extraction

The oil used for the study was extracted from crushed melon seeds in a Soxhlet apparatus using hexane (60-80°C) as a solvent. The extract was desolventized using a rotary-evaporator, stripped and dried in a hot air oven at 120°C for one hour, allowed to cool room temperature. The oil was stored in a refrigerator at about 4^{o}C until it was used.

2.3 Irradiation of Oil Samples

Two grams of the stripped oil was dissolved in 10% methanol in dichloromethane and transferred into an impinger. The impinger was immersed in an ice-water bath and irradiated externally with a 200-watt flood light. The progress of the reaction was monitored by thin layer chromatography (TLC) and reaction with triphenylphosphine, potassium iodide/ starch indicator and sodium thiosulphate and iron(II) sulphate/ammonium thiocyanate solution. A similar experimental set-up was done for another

sample of the oil and kept in the dark as a control.

2.4 Qualitative Detection of Peroxides in the Irradiation Mixture

The reaction mixture and control sample were qualitatively analyzed for their peroxide content by reaction with the following reagents: triphenylphosphine, potassium iodide/ starch indicator and sodium thiosulphate, iron (II) sulphate/ ammonium thiocyanate solution.

2.5 Reaction with Triphenylphosphine

One cubic centimeter of each reaction mixture was transferred into sample bottles and a few crystals of triphenylphosphine were added. The sample bottles were kept in the dark for two hours and monitored by thin layer chromatography.

2.6 Reaction with Potassium Iodide and Starch Indicator

A few crystals of potassium iodide were added to 1 cm^3 of the reaction mixture in sample bottles. The sample bottles were kept in the dark for two hours. Starch indicator was added followed by sodium thiosulphate solution and any colour change(s) were noted.

2.7 Iron (II) Sulphate and Ammonium Thiocyanate

A few crystals of iron (II) sulphate crystals were added to 1 cm^3 of each reaction mixture in sample bottles and kept in the dark for two hours. At the end of two hours, a few crystals of ammonium thiocyanate were added any change in colour was noted.

2.8 Spectral Analysis

FTIR, ^1H and ^{13}C NMR and Distortionless Enhancement Polarization Transfer (DEPT-135) of the oil were run to determine some components of the oil.

3. RESULTS

After column chromatographic purification (stripping) of the extracted oil, TLC analysis, FTIR, ^1H NMR and ^{13}C NMR spectroscopic analyses showed the oil to be pure. The FTIR spectrum of the unoxidized oil (Fig. 1) displayed the following absorptions: 3584.37 (carbonyl ester overtone), CH stretch of cis-olefin 3009.78, 2923.65 and 2854.16 (symmetrical and unsymmetrical vibrations of CH_2 and CH_3), 1744.26 (carbonyl ester), 1464.59 (bending vibrations of CH_2 & CH_3) 1377.84 (bending vibrations of CH_2), 1160.45 (C-O of ester).

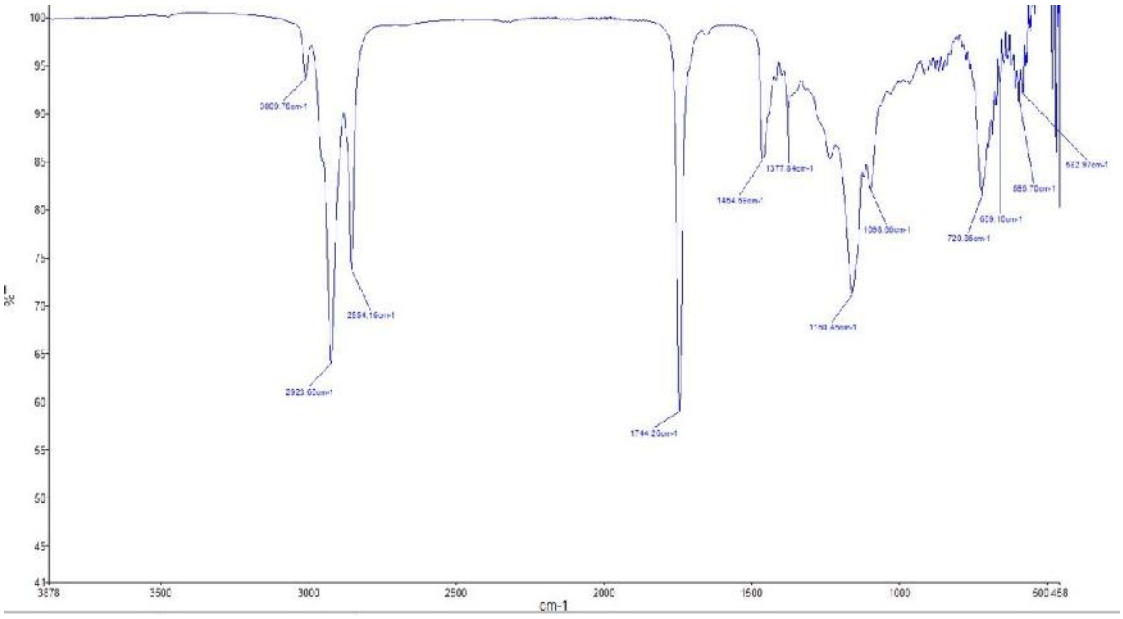

Fig. 1. FTIR spectrum of melon seed oil

The ^{1}H NMR spectrum (Fig. 2) displayed signals between 0.80 and 5.50 ppm. The major signals were a doublet (0.874, 0.861), a triplet (0.851, 0.847, 0.836). Signals of the methylene envelope (1.276, 1.273, 1.227, 1.588, 1.581, 1.567), two doublets (2.040& 2.026; 2.012 & 1.999), two triplets (2.300, 2.294, 2.288 & 2.279, 2.270, 2.264), one triplet (2.753, 2.739, 2.726), two doublets (4.133, 4.121 & 4.109, 4.097), two doublets (4.283, 4.274 & 4.254, 4,251), a multiplet (5.243, 5.235, 5.226, 5.223, 5.214), a multiplet (5.328, 5.317, 5.310, 5.306, 5.296, 5.289, 5.282, 5.275, 5.268, 5,255, 5.246).

The ^{13}C NMR spectrum (Fig. 3) displayed signals between 13.00 and 174 ppm grouped into four regions. 13.99 and 14.03 (terminal methyl carbons), 23 signals between 22.0 and 35 ppm indicating the carbon atoms of the methylene envelope (22.52, 22.64, 24.81, 24.84, 25.61, 27.16, 29.01, 29.05, 29.08, 29.13, 29.23, 29.31, 29.43, 29.48, 29.58, 29.63, 29.66, 29.73, 31.49, 31.88, 33.99, 34.01, 34.15. The glyceryl carbons at 68.91 and 62.07, six signals of between 127 and 130 ppm indicating the vinyl carbons (127.88, 128.06, 129.66, 129.92, 129.94, 130.16), three signals between 170 and 174 indicating the carbonyl carbons (172.79 Sn-2 carbon; 173.21, 173.25, Sn-1,3 carbons). The DEPT-135 spectrum (Fig. 4) showed all the carbon signals in the ^{13}C NMR except those above 170 ppm. Also, carbon atoms bearing odd number of hydrogen atoms (methyl and methine carbons) appear as positive signals while the carbon atoms bearing even number of hydrogen atoms (methylene carbons) appear as negative signals.

4. DISCUSSION

The FTIR spectrum of the unoxidized oil displayed the following absorptions: 3584.34 (carbonyl ester overtone), 3009.78 (C-H stretch of cis olefin), 292365 and 2854.16 (symmetrical and unsymmetrical vibrations of CH_2 and CH_3), 1744.26 (carbonyl of ester), 1464.59 (bending vibrations of CH_2 and CH_3), 1377.84 (bending vibrations of CH_2), 1160.45 (C-O of ester). The overall absorptions portray the oil as containing TAGs with unsaturated acyl chains [9]. The absence of any absorption around 1710 indicates the oil has not undergone any appreciable hydrolytic rancidity [10].

The proton NMR spectrum of the oil (Fig. 2) displayed signals between 0.8 and 5.5 ppm, the usual region of signals for triacylglycerols (TAGs). The signals due to the terminal methyl protons were observed between 0.874 and 0.836 ppm. The allylic and bis-allylic protons of the

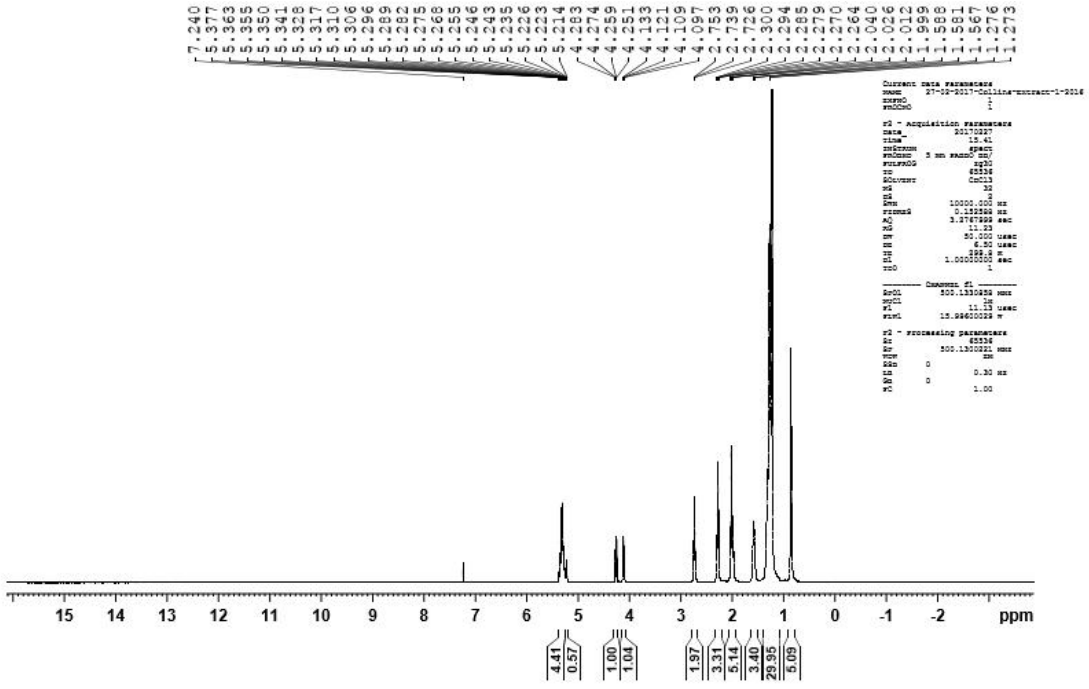

Fig. 2. Proton NMR spectrum of melon seed oil

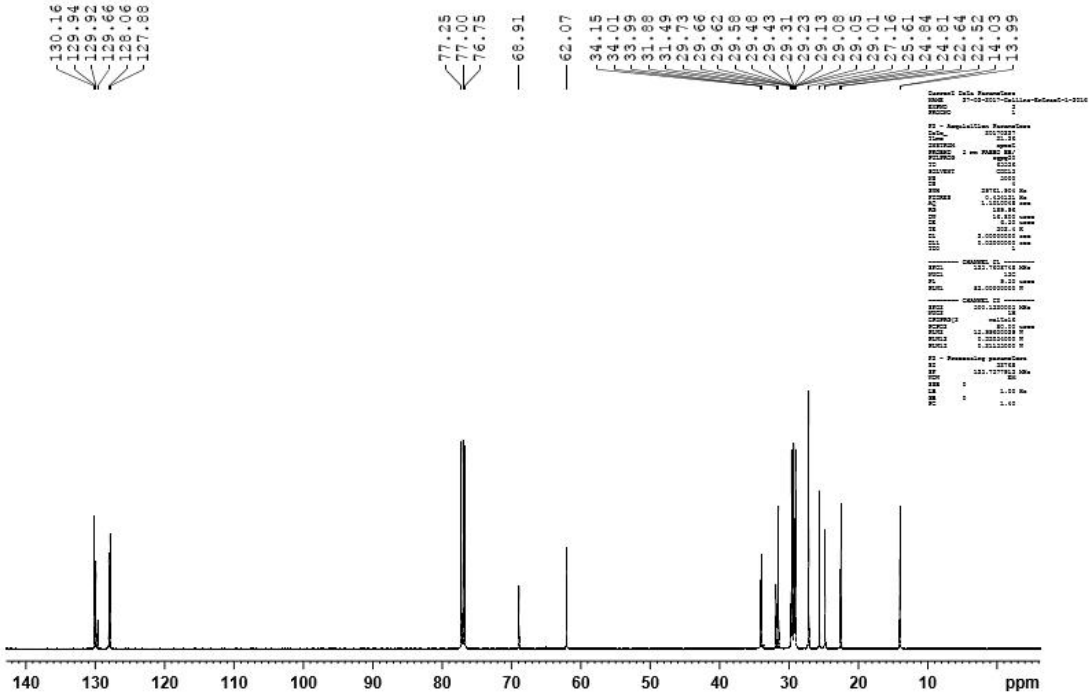

Fig. 3. ^{13}C NMR spectrum of melon seed oil

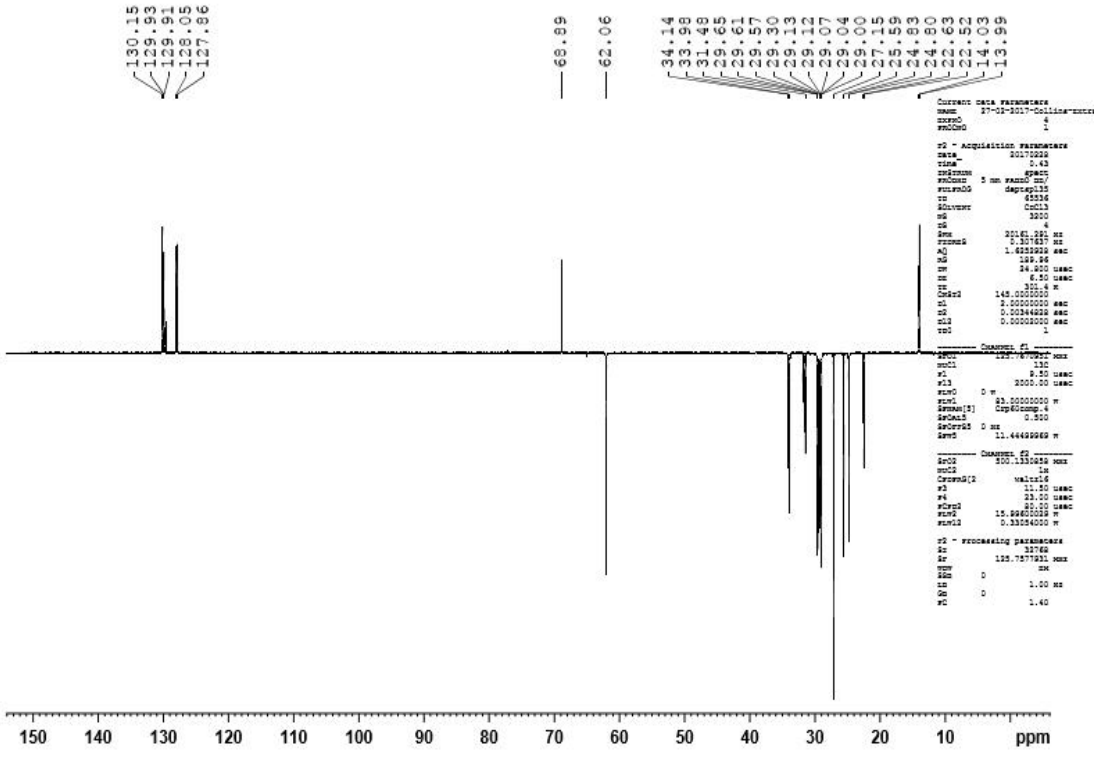

Fig. 4. DEPT-135 spectrum of melon seed oil

unsaturated acyl groups were observed as a double doublet (d,d) at 1.999, 2.012, 2.026 and 2.040 and a triplet (t) observed at 2.726, 2.739 and 2.753 ppm respectively. These signals confirm the presence of oleyl, linoleyl and linolenyl acyl groups in the TAGs of the oil. The triplet signals at 1.567, 1.581 and 1.588 were due to the protons of the methylene groups beta to the carbonyl groups. On the other hand, the protons of the methylene groups alpha to the carbonyl groups were observed between 2.264 and 2.300 as a triplet. Two multiplets between 4.097 and 4.283 represent the signals due to the Sn- 1,3 protons of the glyceryl group, while the signals between 5.214 and 5.255 ppm were due the Sn-2 protons of the glyceryl group. The multiplet between 5.265 and 5.328 ppm were signaled due to the vinyl protons. These assignments were in comparison with published literature [7,11].

The ^{13}C NMR spectrum (Fig. 3) displayed signals ranging between 14.0 and 174 ppm grouped into four regions according to the different types of carbon atoms in the acyl chains of TAGs. The signals were assigned chemical shifts by comparison with published literature [12,13]. The signals due to the saturated carbon were observed between 14 and 30 ppm. Those at 14 and 14.1 ppm were assigned to terminal methyl carbons, while the signals between 20 and 40 were due to methylene carbons of the acyl chains. The peaks at 27.17 and 27.18 were due to allylic carbons of linoleic, oleic and linolenic acids, while the signal at 25.2 is due to the bis-allylic carbons of linoleic and linolenic acids. The signals due to the glyceryl carbons were observed at 62.02 (Sn) and 68.86 (Sn-) ppm. The signals between 120 and 130 were due to the vinyl carbons and the signals between 170 and 180 were assigned to the carbonyl carbons [14]. These assignments were further confirmed by deductions from the DEPT-135 spectrum (Fig. 4). The methine carbons of the glyceryl group, the vinyl carbons and the terminal methyl carbons were observed as positive signals while the methylene carbons of the acyl chain and the glyceryl group were observed as negative signals [15]. The signals above 170 ppm earlier observed in the ^{13}C NMR spectrum were not observed in the DEPT-135 confirming these signals as due to carbonyl (quaternary) carbons [16].

The irradiated reaction mixture reacted with the test reagents: triphenylphosphine, potassium iodide/ starch indicator and sodium thiosulphate, iron (II) sulphate/ ammonium thiocyanate solution. The results indicate that oxidation products were formed in the photooxidation reaction since the test reagents are reducing agents. Triphenylphosphine reacted rapidly to give products as indicated by TLC analysis. The amount of triphenylphosphine oxide produced has been used as the basis of peroxide value determination of oxidized oils using methods such as electron-ionization spectrometry [17], measuring the absorbance of triphenylphosphine oxide(TPPO) produced using FTIR spectroscopy at 532 cm^{-1} [18]. In a related procedure, Li et al. [19], used the amount of TPPO produced to determine the peroxide value in thermally oxidized crude palm oil and edible oils respectively using Near IR spectroscopy. Talpur, et al. [20], used UV-Visible spectroscopy to measure the absorbance of TPPO at 240 nm, which is a measure of the amount of hydroperoxide present in the oxidized oil.

The photooxidation reaction mixture reacted with potassium iodide to give a brown solution which on an addition of starch indicator turned blue-black. This reaction is the basis of the AOCS iodometric determination of peroxide value of oxidized oils [21]. It is adapted here as a qualitative test for peroxides.

In the reaction with iron (II) sulphate and ammonium thiocyanate, after the incubation period and subsequent addition of the thiocyanate crystals, a blood-red colour was observed. This reaction is the basis for the International Dairy Federation (IDF) method of peroxide value determination of oxidized oils. [22,23,24].

5. CONCLUSION

The results of the spectral analysis showed that melon seed oil contains unsaturated TAGs (oleyl, linoleyl and linolenyl acyl chains). This confirms the ease of sensitized oxidation and nutritional importance of the oil due to the presence of omega-3 and omega-6 fatty acids in the oil. The results of sensitized oxidation further confirm the unsaturated nature of the TAGs in the oil. Chemical and TLC analyses indicate the presence of peroxides in the irradiation reaction mixture. The ease of oxidation via sensitized oxidation suggests that the oil should be stripped after production for a longer shelf-life.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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